Theoretical and Applied Genetics 45, 315–321 (1975)

Alterations in Mitochondria Associated with Cytoplasmic and Nuclear Genes Concerned with Male Sterility in Maize

D. H. P. BARRATT and R. B. FLAVELL

Cytogenetics Department, Plant Breeding Institute, Cambridge (England)

Summary. Mitochondria isolated from etiolated shoots of a range of maize genotypes with the "Texas" cytoplasm conferring cytoplasmically-inherited male sterility, are sensitive to a pathotoxin isolated from *Helminthosporium maydis*, race T. The pathotoxin inhibits oxidation of α ketoglutarate and malate and stimulates NADH oxidation. The time taken for the pathotoxin to induce these changes is a measure of the sensitivity of the mitochondria to the pathotoxin. A range of nine different pairs of genotypes, each pair differing principally in the presence of nuclear male fertility restorer alleles has been compared in their sensitivity to pathotoxin. In every case the line carrying the restorer alleles is more resistant to the pathotoxin. The restored genotypes can be quantitatively arranged into groups which correspond to the four different sources of the restorer genes in these lines. It is suggested that the restorer genes cause changes in mitochondria, which modify the functional aberration introduced by the cytoplasmically-inherited mutation causing sterility.

Introduction

A common form of male sterility in plants is inherited via the cytoplasm. Cytoplasmically-inherited male sterility has been reported in some 80 species, 25 genera and 6 families (Edwardson, 1970). Various hypotheses have been put forward to explain the genetic origin of the sterility: (1) a viral or episome-like infection occurs in the pollen which "sterilises" the pollen cytoplasm (Frankel, 1956, 1962, 1964; Edwardson and Corbett, 1961; Bianchi, 1963; Curtis, 1967 and Ohta, 1967); (2) a "foreign" cytoplasm is introduced by an interspecific or intergeneric cross at some time (Edwardson, 1970); (3) cytoplasmic mutations are induced in the presence of particular nuclear genotypes (Rhoades, 1950); and (4) spontaneous mutations occur in cytoplasmic DNAs (Duvick, 1965).

Support for the idea that the sterility could result from changes within the plant cytoplasmic DNA came with the proof that mitochondria and chloroplasts contain DNA essential for their structure and function (Nass, 1969; Taylor, 1970) and that mutations in their DNAs cause phenotypic aberrations that are cytoplasmically inherited (Sager, 1972). Some recent biochemical and electron microscopical observations have also suggested male sterility is correlated with alterations in mitochondria. Electron microscopy of the fine structure of maize anthers has shown that the introduction of nuclear fertility restorer genes partially restores the aberrant structures of the cytoplasmic organelles, especially the mitochondria, seen in cytoplasmic male sterile lines (Turbin *et al.*, 1968). Similar studies on wheat anther tissue have shown that in male sterile sporogenous tissue and pollen there are fewer organelles and these appear inactive and subject to rapid degeneration (De Vries and Ie, 1970). Also, mitochondria isolated from male sterile wheat seedlings were found to have a higher oxidative phosphorylation efficiency than mitochondria from male fertile seedlings (Srivastava, Sarkissian and Shands, 1969).

In Texas in 1944, J. S. Rogers discovered a source of cytoplasmically-inherited male sterility in maize (Duvick, 1965). By 1970 "Texas" male sterile cytoplasm (Tcms) was being used to produce about 85% of the hybrid maize seed in the United States (Ullstrup, 1972), complete restoration of fertility being achieved by inclusion of nuclear, dominant alleles at the Rf_1 and Rf_2 loci. In 1969, 1970 and 1971 there was an epiphytotic outbreak of Southern Corn Leaf Blight in North America caused by *Helminthosporium maydis* Nisikado and Miyake, race T. This race was highly specific in pathogenicity to inbreds and hybrid plants with Tcms. Plants with "normal" cytoplasm were resistant (Hooker *et al.*, 1970a, b). A pathotoxin can be isolated from cultures of *Helminthosporium maydis*, race T or from infected leaves which will induce the disease symptoms when injected into plants with Tcms but not plants with "normal" cytoplasm (Hooker *et al.*, 1970a, b; Turner and Martinson, 1972; Gracen, Grogan and Forster, 1972). Using this pathotoxin, the mitochondria from seedlings with Tcms have been shown to be different from those in seedlings with the same nuclear genetic background but with normal, male fertile cytoplasm (Miller and Koeppe, 1971). In mitochondria from "Texas" seedlings the pathotoxin causes uncoupling of oxidative phosphorylation, irreversible swelling in

KCl medium, inhibition of the electron transport chain before cytochrome b and activation of ATPase in the presence of oxidisable substrates (Miller and Koeppe, 1971; Peterson, Flavell and Barratt, 1975; Flavell, 1974a). Mitochondria from seedlings with normal cytoplasm, however, are completely resistant to the pathotoxin. Because the pathotoxin affects activities associated with the inner mitochondrial membrane, it was suggested that the "Texas" cytoplasm contains a genetic modification that produces an altered inner mitochondrial membrane able to bind pathotoxin.

Cytoplasmic male sterility in maize can be completely suppressed by a combination of two or three different nuclear restorer alleles (Duvick, 1965). If the cause of male sterility in "Texas" cytoplasm is somehow related to the alteration in the mitochondria then it seemed likely that the restorer alleles might partially correct or at least modify the mitochondrial membrane sensitivity to *Helminthosporium maydis* pathotoxin. We report here the effects of nuclear male fertility restorer alleles in maize on inner mitochondrial membrane sensitivity to *Helminthosporium maydis* race T pathotoxin.

Materials and Methods

Maize genotypes used: The genotypes used and their sources are given in table 1. The seeds were either from a foundation source (F), or from a hand pollinated source (H). Foundation seeds were from T × N crosses (T = male sterile line, N = maintainer line). Although the seed sources of "Texas" cytoplasm are noted as different they go back to a single origin. There are four sources of the restorer alleles in the stocks we used. The restorer alleles (designated *Rf Rf*) were transferred into the nuclear backgrounds of the different male sterile lines by a backcrossing programme selecting for restoration in each generation. The numbers of backcrossing and selfing generations are shown in table 1. All lines were essentially isogenic, based on field appearance and on a trial in which F_1 hybrids were tested for heterosis. In no case was any difference in the residual genotype of the line noted (P. A. Peterson, personal communication). The $F7^T \times F2$ male sterile hybrid seed was kindly supplied by Dr. Kiss, Hodee Maïs Angevin, La Menitre, France.

Growth of seedlings and isolation of mitochondria

Maize seeds were surface sterilised for 15 minutes in 2% sodium hypochlorite, thoroughly washed by tap water for 3 hours, and then spread evenly on deionised water-saturated paper towels. Following inhibition at 5 °C for 2 days the seeds were germinated in the dark at 27 °C for 3 to 5 days depending on the rate of germination and rate of growth. All subsequent operations were carried out at 1 – 2 °C using pre-chilled apparatus. The required weight of etiolated shoots was ground in a mortar in 22 ml of ice-cold grinding medium for 35 seconds. The grinding medium consisted of .5M mannitol, 10mM TES pH 7.2, 1mM EGTA, 0.2% BSA and 0.05% cysteine.

The homogenate was allowed to drip through 4 layers of close-weave terylene fabric and the resulting filtrate centrifuged up to 12,000 × g and then rapidly stopped. The supernatants were centrifuged at 30,000 × g for 1 minute. A small starch pellet within the large mitochondrial pellet was carefully removed with a spatula. Eight ml of fresh grinding medium without cysteine were poured carefully down the sides of each of the tubes and then decanted. The walls of the centrifuge tubes were wiped and the mitochondrial pellets were dispersed in either 1.2 or 0.8 ml of assay medium depending on whether NADH or α ketoglutarate was to be used as oxidisable substrate. The assay medium consisted of .3M mannitol, 10 mM TES pH 7.2, 10 mM potassium phosphate pH 7.2, 1 mM EGTA, 10 mM KCl, 5 mM $MgCl_2$ and 0.75 mg/ml BSA. The day-to-day standardisation of the yield and quality of mitochondrial preparations was maintained by a strict and accurate adherence to the fresh weight of shoots used and the quantities of grinding and suspension media. Independent tests confirmed that replicate extractions produced mitochondrial preparations with very similar protein content and ADP/O ratios, (ADP/O approximately 3.0 with α ketoglutarate as substrate).

Mitochondrial assays

Oxygen uptake was determined at 23 °C with an oxygen electrode (Rank Bros. Bottisham, Cambs), in a total reaction volume of 1.5 ml (0.6 ml mitochondrial suspension + 0.9 ml assay medium). Either α ketoglutarate (10mM) or NADH (1 mM) was added as substrate and

Table 1. *Source of the genotypes*

Genotype	Source of seed			History of T *Rf Rf* lines		
	Normal	T *rf rf*	T *Rf Rf*	No. backcrosses	No. selfs	Source *Rf*
B37	JO597 (F)*	Downey 67 (F)	Pelee 64 (F)[a]	ISU		BSSS-Ky21
C103	113084+ (H)+	PC Tipton 62 (F)	Pelee 62 (F)	6	3	KY21
M14	Pelee 61 (F)	410202 (H)	117206 (H)	6?	8	BH2
B14	Pelee 65 (F)	Pelee 65 (F)	PC Tipton 65 (F)	7	4	BH2
OH43	Pelee 68 (F)	Pelee 68 (F)	Polk 67 (F)	9	3	F5DD1
A73	PC C6393 (F)	Pelee 60 (F)	Johnston 63 (F)	8	4	F5DD1
A632	CO319 (F)	Polk 69 (F)	Clyde Black & Sons (F)	B		W153R
W64A	T79266+ (H)	Polk 67 (F)	Pelee 67 (F)	B		W153R
H84	JO887 (F)	PC Tipton 69 (F)	HF001032+ (H)	8	4	W153R

(F)* = Foundation seed
(H)+ = Hand Pollinated
B = Clyde Black & Sons, a Foundation Seed Co., no data.
ISU = Iowa State University, no data

[a] Five other sources of this genotype were also tested

State 3 respiration was initiated by the addition of ADP (0.2 mM). Following termination of State 3 respiration, pathotoxin was added in amounts ranging from 1 to 10 µl. Subsequent cycles of State 3 respiration, if the pathotoxin had no effect, were brought about by the addition of further quantities of ADP. If the pathotoxin altered the oxygen uptake rate, the times taken to uncouple NADH oxidation or inhibit α ketoglutarate oxidation were measured. With α ketoglutarate as substrate approximately 0.39 mg of mitochondrial protein was present in the assay and with NADH as substrate approximately 0.26 mg mitochondrial protein.

The effect of the pathotoxin on mitochondrial oxidation of malate without exogenous NAD^+, using 2, 6 dichlorophenolindophenol as electron acceptor, was determined on the same mitochondrial suspensions as those used for the oxygen electrode studies. Malate oxidation was assessed at 35 °C from the rate of optical density decrease at 600 nm in a 1.3 ml reaction mixture consisting of 1.0 ml assay medium, 0.1 ml of 0.6×10^{-5} M 2, 6 dichlorophenolindophenol, 0.1 ml of 0.02 M sodium azide, 0.05 ml of 1.22 M sodium malate (pH 6.8) and 0.05 ml of mitochondrial suspension. The concentration of mitochondria in the final assay produced Δ OD 600 nm of approximately 0.1 per minute. Pathotoxin in varying amounts was added about 1 minute after the reaction had started and the time to inhibit malate oxidation completely was determined.

Pathotoxin Preparation

This was a gift from Dr. Carl Tipton (Tipton, Mondal and Uhlig, 1973). Concentrations were used which enabled different genotypes to be distinguished as described in Results.

Results

Mitochondria were isolated from etiolated shoots of groups of closely related genotypes, each group containing two lines with the same nuclear genotype but with different cytoplasmic genotypes (N or T) and a third line with T cytoplasm and the same genetic background as the other two lines but with homozygous nuclear male sterility restorer alleles substituted. The effect of the *H. maydis* race T pathotoxin on oxidative phosphorylation and respiration within these mitochondria was quantitatively studied using oxygen and 2, 6 dichlorophenolindophenol (DCIP) as electron acceptors.

Mitochondria isolated from all of the genotypes with the normal N cytoplasm were insensitive to the pathotoxin in all assays used. The addition of pathotoxin to almost all preparations of mitochondria from plants carrying the T cytoplasmic genome caused the stimulation and uncoupling of NADH respiration and inhibition of α ketoglutarate oxidation (See accompanying paper: Peterson, Flavell and Barratt, 1975; Miller and Koeppe, 1971). The inhibition of malate oxidation within the malate dehydrogenase — NADH dehydrogenase complex of the electron transport chain using DCIP as an electron acceptor is illustrated in Fig. 1. The time taken for the pathotoxin to inhibit malate oxidation completely is related to the pathotoxin concentration and is a useful estimate of the sensitivity of a mitochondrial preparation to the pathotoxin (Flavell, 1974a). This parameter was

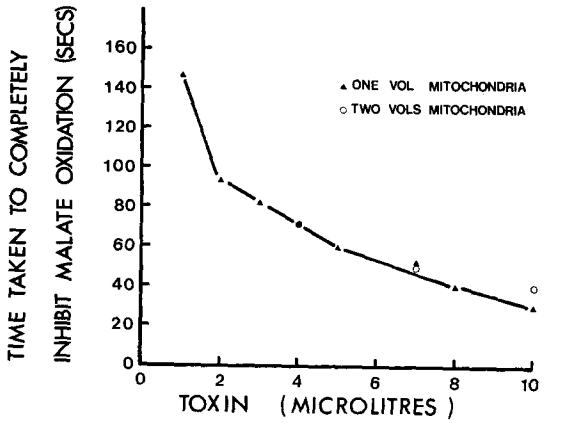

Fig. 1. Relationship between pathotoxin concentration and the time for complete inhibition of malate oxidation in mitochondria
Mitochondria from the $F7^T \times F2$ male sterile hybrid were incubated with malate and malate oxidation measured using 2, 6 dichlorophenolindophenol (DCIP) as an electron acceptor. Different volumes of pathotoxin were added and the time for complete cessation of DCIP reduction was measured

essentially independent of mitochondrial protein concentration over the concentrations used (Fig. 1). An *in vitro* mixing experiment was carried out with the sensitive mitochondria from B37 T *rf rf* shoots and the resistant mitochondria from B37 N shoots. The 1:1 mixture was sensitive to pathotoxin to an intermediate extent, the biphasic curve indicating a mixed population of mitochondria and the absence of soluble factors in one of the mitochondrial preparations being responsible for the differential sensitivities (Fig. 2).

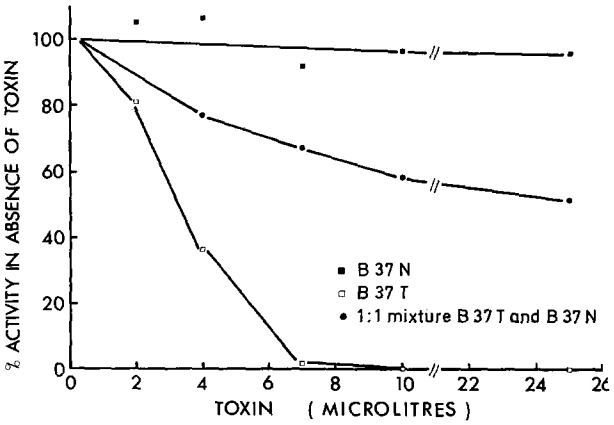

Fig. 2. Effect of pathotoxin on malate oxidation in mitochondria from B37 T *rf rf* and B37 N and a 1:1 mixture of these mitochondria
Malate oxidation was assayed as in Figures 1 and 3. The rates of DCIP reduction between 1 min 40 sec and 2 min after addition of the pathotoxin were determined and the percentage inhibition calculated from a control incubation lacking pathotoxin

The time taken for the mitochondria to become uncoupled, as indicated by the rate of oxygen uptake, was also similarly related to the pathotoxin concen-

Table 2. *Stimulation of NADH respiration by pathotoxin in mitochondria from T rf rf and T Rf Rf genotypes*

Genotype (Source of *Rf*)	Toxin added ml × 10^{-3}	Time taken to stimulate respiration after addition of toxin (secs)		T *Rf Rf**
		T *rf rf*	T *Rf Rf*	T *rf rf*
B37(BSSS-KY21)	2	11	no stimulation	∞
	10	9	no stimulation	∞
C103(KY21)	2	9	33	3.7
M14(BH2)	1	14	31	2.3
	2	11	27	2.4
B14(BH2)	1	15	30	2.0
	2	11	21	2.0
OH 43(F5DD1)	10	9	13	1.4
	10	8	13	1.5
A 73(F5DD1)	1	13	21	1.6
	2	10	16	1.6
A632(W153R)	1	11	14	1.3
	2	9	12	1.3
W64A(W153R)	1	12	17	1.4
	2	8	12	1.4
H84(W153R)	2	11	13	1.2

* Ratio of time taken to stimulate mitochondrial respiration of T *Rf Rf* compared with T *rf rf* for same amount of pathotoxin. Mitochondria from N cytoplasm for each of the above groups of genotypes were tested and found to be completely resistant to pathotoxin

Table 3. *Inhibition of α ketoglutarate oxidation by pathotoxin in mitochondria from T rf rf and T Rf Rf genotypes*

Genotype (Source of *Rf*)	Toxin added ml × 10^{-3}	Time taken to commence inhibition of respiration after addition of toxin (secs)		T *Rf Rf**
		T *rf rf*	T *Rf Rf*	T *rf rf*
B37(BSSS-KY21)	2	33	no uncoupling or inhibition	∞
	10	8	no uncoupling or inhibition	∞
C103(KY21)	2	45	151	3.4
M14 (BH2)	2	31	114	3.7
	2	29	111	3.9
B14 (BH2)	1	98	uncoupling no inhibition	∞
	2	48	135	2.8
OH 43(F5DD1)	10	14	31	2.2
	10	10	21	2.2
A73(F5DD1)	1	50	87	1.8
	2	39	69	1.8
A632(W153R)	1	50	62	1.2
	2	34	40	1.2
W64A(W153R)	1	44	66	1.5
	2	29	44	1.5
H84 (W153R)	2	34	46	1.4

* Ratio of time taken to inhibit mitochondrial respiration of T *Rf Rf* compared with T *rf rf* for same concentration of pathotoxin. Mitochondria from seedlings with N cytoplasm in each of the above groups of genotypes were tested and found to be completely resistant to pathotoxin

tration and sensitivity of the mitochondria to the pathotoxin. Thus to estimate mitochondrial sensitivity in the studies with oxygen and DCIP as electron acceptors, the times taken to uncouple NADH oxidation or inhibit α ketoglutarate or malate oxidation with pathotoxin were measured. The times taken for mitochondria from pairs of genotypes, with members of each pair differing in their nuclear restorer alleles, to respond to the pathotoxin in the oxygen electrode chamber during the oxidation of NADH and α ketoglutarate are shown in tables 2 and 3 respectively. All the mitochondria from lines with the homozygous male fertility restorer alleles required a longer exposure to pathotoxin before they reacted than did their counterparts from lines lacking the male fertility restorer alleles. The ratio of the time taken to inhibit or stimulate respiration, depending on substrate, in mitochondria from T *Rf Rf* restored genotypes and the time taken in mitochondria from T *rf rf* male sterile genotypes for the same amount of pathotoxin, is included as a convenient summary of the consequence of the restorer alleles on pathotoxin sensitivity. The sensitivities of mitochondria from restored T *Rf Rf* lines, compared with those from sterile T *rf rf* lines, can be roughly divided into four groups corresponding to the four sources of the restorer alleles. The resistance varied from complete in the case of B37 T *Rf Rf* to very little for H84 T *Rf Rf*. When the mitochondria from B14 T *Rf Rf* were incubated with α ketoglutarate and a low concentration of pathotoxin, the pathotoxin prevented increased respiration on the addition of ADP (State 3 respiration) but there was no inhibition of State 4 respiration, indicating, as detailed elsewhere (Peterson, Flavell and Barratt, 1975), that pathotoxin prevents State 3 respiration prior to and at a lower pathotoxin concentration than inhibition of State 4 respiration.

The times taken for different doses of pathotoxin to inhibit malate oxidation completely in mitochondria isolated from these same groups of genotypes are shown in Fig. 3. As with the estimates on rates of oxygen uptake, all the mitochondria from lines bearing the restorer alleles were more resistant to pathotoxin than their counterparts lacking the restorer alleles. The resistance of the mitochondria could be roughly grouped as before on the basis of the four sources of the restorer alleles.

Between the different groups of genotypes, overall differences in mitochondrial pathotoxin sensitivity were apparent (tables 2 and 3, Fig. 3) indicating that the different nuclear genetic backgrounds also affected mitochondrial sensitivity to pathotoxin.

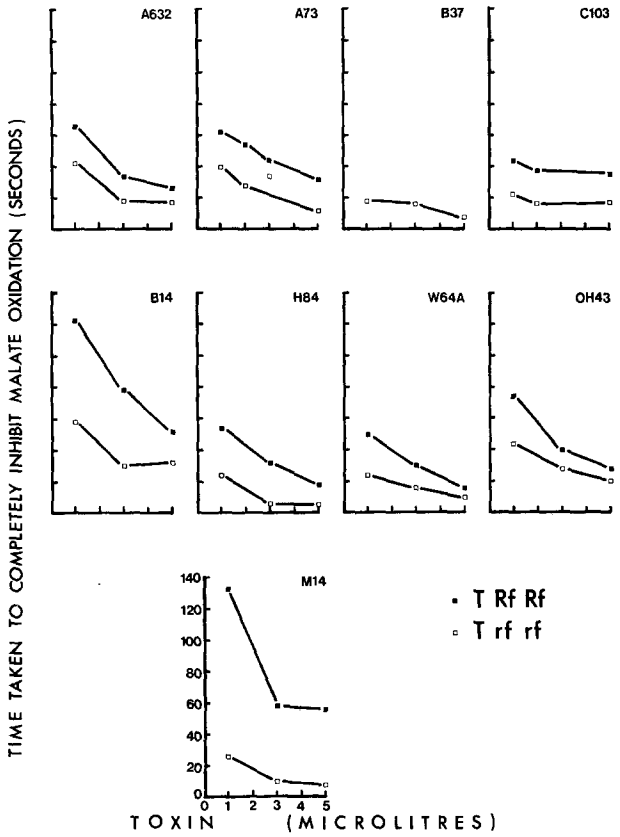

Fig. 3. Alteration of the sensitivity of malate oxidation to pathotoxin in the presence of restorer alleles
Mitochondria from each of the indicated genotypes were incubated with malate and malate oxidation measured using 2, 6 dichlorophenolindophenol (DCIP) as an electron acceptor. Different volumes of pathotoxin were added and the time taken to inhibit completely DCIP reduction measured. Malate oxidation in mitochondria from lines with normal cytoplasm and from 6 different sources of B37 T *Rf Rf* were completely resistant to pathotoxin

Discussion

The results reported in this paper illustrate that maize plants with cytoplasmically-inherited "Texas" male sterility possess apparently altered mitochondria as judged by their sensitivity to the pathotoxin prepared from *Helminthosporium maydis*, race T (Fig. 1–3, tables 2 and 3; Miller and Koeppe, 1971; Peterson, Flavell and Barratt, 1975; Flavell 1974a). Furthermore, from our observations on both uncoupling of oxidative phosphorylation and inhibition of electron transport by the pathotoxin in a range of nine groups of genotypes we can conclude that the presence of male fertility restorer alleles in the nucleus modifies this sensitivity to the pathotoxin (tables 2 and 3, Fig. 2 and 3). This is additional evidence to support the hypothesis that altered mitochondria are involved in the mechanism of cytoplasmic male sterility. It also suggests, but by no means proves, that the products of male fertility restorer genes may be mitochondrial inner membrane proteins. A model for the mechanism of cytoplasmic male sterility incorporating these results has recently been proposed (Flavell, 1974b).

The reductions in pathotoxin sensitivity of the mitochondria in the presence of the restorer alleles can be devided into four groups which correspond with the four different sources of the restorer alleles. The alleles at the major restorer loci could be different in the four sources of the dominant restorer alleles or the different sources may possess different restorer modifiers (Duvick, 1965) which have been selected with the major restorer alleles during the backcrossing programmes. We cannot, of course, discount the possibility that the variation in mitochondrial sensitivity comes from genes closely linked to the *Rf* loci, rather than from the *Rf* loci themselves (Russell and Marquez-Sanchez, 1966). Extensive genetic analyses would be required to clarify this. However, that the changes in the inner mitochondrial membrane are due to the restorer alleles seems likely since they are partial functional reversions of the original change associated with sterility.

Whether a correlation exists between the mitochondrial measurements of the effects of the restorer alleles and restoration of pollen fertility requires data on the fertility of these genotypes in a uniform environment. A correlation is not necessarily expected since the mitochondrial modification conferring sensitivity to pathotoxin is unlikely to be at the same location as the alteration resulting in male sterility; mitochondria from other male sterile cytoplasms are insensitive to the pathotoxin (Gracen, Forster and Grogan, 1971; Smith *et al.*, 1971). It is more likely that changes in pathotoxin sensitivity come about by conformational changes within the mitochondria, induced by the presence of the restorer gene products at other sites on the inner membrane.

An interaction between nuclear and cytoplasmic genes at the phenotype level; i. e., in restoration of pollen fertility, as well as at the organelle structure level, is in complete agreement with the known roles of cytoplasmic DNAs. The function and regulation of mitochondrial and chloroplast gene products are dependent upon the function and regulation of nuclear gene products and *vice versa* (Sager, 1972; Taylor, 1970). Nuclear gene suppressors of cytoplasmic mutants which cause mitochondrial aberrations are known in *Neurospora* (Mitchell and Mitchell, 1956). Further evidence indicating nuclear/cytoplasmic interactions is the variation in mitochondrial sensitivity to pathotoxin in different nuclear backgrounds (tables 2 and 3, Fig. 3).

There are many, presumably different, cytoplasms in addition to Tcms that confer male sterility on maize (Beckett, 1971). The majority, but not all, are resistant to *Helminthosporium maydis* race T (Gracen, Forster and Grogan, 1971; Smith *et al.*, 1971). However, susceptibility is dependent on nuclear genotype (Gracen and Grogan, 1972; Hooker, 1971). The relationships of these cytoplasms to one another and whether they contain mitochondrial differences similar to those in "Texas" cytoplasm are not known but are currently being investigated.

The relationship between plant sensitivity to *Helminthosporium maydis* race T and mitochondrial sensitivity to *Helminthosporium maydis* race T pathotoxin is a strong indication that mitochondrial sensitivity is the basis of susceptibility of maize lines with Tcms to the fungal disease. However, observations on the effect of the pathotoxin on plant tissues suggest the biochemical basis of disease susceptibility may be more complex (Arntzen *et al.*, 1973; Tipton, Mondal and Uhlig, 1973; Peterson, Flavell and Barratt, 1975). Mitochondria from etiolated seedlings with Tcms and restorer alleles were slightly more resistant to pathotoxin, but only the B37 T *Rf Rf* sample had resistant mitochondria. It is known that sensitivity of Tcms lines to *Helminthosporium maydis* race T is dependent on nuclear genes but most strains with restorer alleles seem as sensitive to the disease as those strains without the restorer alleles (Berquist and Peverley, 1972; Hilty and Josephson, 1971; Gracen and Grogan, 1972).

Further careful work with well characterised maize genotypes, fungal isolates and better purified pathotoxin preparations is necessary before the effect of restorer alleles on disease susceptibility can be critically evaluated and compared with the changes in mitochondrial sensitivity that we have demonstrated here.

Acknowledgements

We wish to express our thanks to Professor Peter Peterson of Iowa State University for his collaboration in supplying us with seeds and a pathotoxin preparation. We thank Dr. Colin Law of this Department for his help in the preparation of the manuscript.

D. H. P. Barratt is the recipient of an Agricultural Research Council Studentship.

Literature

Arntzen, C. J., Koeppe, D. E., Miller, R. J., Peverly, J. H.: The effect of pathotoxin from *Helminthosporium maydis* (race T) on energy-linked processes of corn seedlings. Physiol. Plant Path. **3**, 79–89 (1973).

Beckett, J. B.: Classification of male sterile cytoplasms in maize (*Zea mays* L.). Crop Sci. **11**, 724–726 (1971).

Berquist, R. R., Peverly, G.: Reaction of corn inbreds and hybrids with different cytoplasms and genotypes to *Helminthosporium maydis*-Race T. Plant Disease Reporter **56**, 112–114 (1972).

Bianchi, F.: Transmission of male sterility in Petunia by grafting. Genen Phaenen **8**, 53–64 (1963).

Curtis, G. J.: Graft transmission of male sterility in sugar beet (*Beta vulgaris* L.) Euphytica **16**, 419–424 (1967).

De Vries, A. P., Ie, T. S.: Electron-microscopy of anther tissue and pollen of male sterile and fertile wheat (*Triticum aestivum* L.). Euphytica **19**, 103–120 (1970).

Duvick, D. N.: Cytoplasmic pollen sterility in corn. Adv. in Genetics **13**, 1–56 (1965).

Edwardson, J. R.: Cytoplasmic male sterility. Bot. Rev. **36**, 341–420 (1970).

Edwardson, J. R., Corbett, M. K.: Asexual transmission of cytoplasmic male sterility. Proc. Nat. Acad. Sci. **47**, 390–396 (1961).

Flavell, R. B.: The inhibition of electron transport by *Helminthosporium maydis* race T pathotoxin in maize mitochondria altered by a cytoplasmically-inherited mutation. Physiol. Plant Pathol. (1974a, in press).

Flavell, R. B.: A model for the mechanism of cytoplasmic male sterility, with special reference to maize. Plant Sci. Letters **3**, 259–263 (1974b).

Frankel, R.: Graft induced transmission to progeny of cytoplasmic male sterility in Petunia. Science **124**, 684–685 (1956).

Frankel, R.: Further evidence on graft induced transmission to progeny of cytoplasmic male sterility in Petunia. Genetics **47**, 641–646 (1962).

Frankel, R.: The nature of cytoplasmic male sterility in Petunias. (English summary). Spec. Bull. 69, Nat. and Univ. Inst. Agric. Rehovot 1–VIII (1964).

Gracen, V. E., Forster, M. J., Grogan, C. O.: Reactions of corn (*Zea mays*) genotypes and cytoplasms to *Helminthosporium maydis* toxin. Plant Disease Reporter **55**, 938–941 (1971).

Gracen, V. E., Grogan, C. O.: Reactions of corn (*Zea mays*) seedlings with non sterile, Texas male sterile, and restored Texas male sterile cytoplasms to *Helminthosporium maydis* toxin. Plant Disease Reporter **56**, 432–433 (1972).

Gracen, V. E., Grogan, C. O., Forster, M. J.: Permeability changes induced by *Helminthosporium maydis*, race T, toxin. Canad. J. of Botany **50**, 2167–2170 (1972).

Hilty, J. W., Josephson, L. M.: Reaction of corn inbreds with different cytoplasms to *Helminthosporium maydis*. Plant Disease Reporter **55**, 195–198 (1971).

Hooker, A. L.: *Helminthosporium maydis* a challenge for the maize breeder. Proceedings of the 6th meeting of the Maize and Sorghum section of Eucarpia 333–367 (1971).

Hooker, A. L., Smith, D. R., Lim, S. M., Beckett, J. B.: Reaction of corn seedlings with male sterile cytoplasm to *Helminthosporium maydis*. Plant Disease Reporter **54**, 708–712 (1970a).

Hooker, A. L., Smith, D. R., Lim, S. M., Musson, M. D.: Physiological races of *Helminthosporium maydis* and disease resistance. Plant Disease Reporter **54**, 1109 to 1110 (1970b).

Miller, R. J., Koeppe, D. E.: Southern corn leaf blight: susceptible and resistant mitochondria. Science **173**, 67–69 (1971).

Mitchell, M. B., Mitchell, H. K.: A nuclear gene suppressor of a cytoplasmically inherited character in *Neurospora crassa*. J. Gen. Microbiol. **14**, 84–89 (1956).

Nass, S.: Similarities of bacteria and mitochondria. Intern. Rev. Cytology **25**, 55–129 (1969).

Ohta, Y.: Effect of thermoshock and virus inoculation on cytoplasmic male sterility in Capsium and Petunia. Ann. Rep. Nat. Inst. Genet. Japan **18**, 26 (1967).

Peterson, P. A., Flavell, R. B., Barratt, D. H. P.: Altered mitochondrial membrane activities associated with cytoplasmically-inherited disease sensitivity in maize. Theor. Appl. Genetics **45**, 309–314 (1975).

Rhoades, M. M.: Gene induced mutation of a heritable cytoplasmic factor producing male sterility in maize. Proc. Nat. Acad. Sci. US. **36**, 634–635 (1950).

Russell, W. A., Marquez-Sanchez, F.: Effect of cytoplasmic male sterility and restorer genes on performance among different genotypes of corn (*Zea mays* L.) Crop Sci. **6**, 294–295 (1966).

Sager, R.: Cytoplasmic genes and organelles. New York: Academic Press 1972.

Smith, D. R., Hooker, A. L., Lim, S. M., Beckett, J. B.: Disease reaction of thirty sources of cytoplasmic male sterile corn to *Helminthosporium maydis* race T. Crop Sci. **11**, 772–773 (1971).

Srivastava, H. K., Sarkissian, I. V., Shands, H. L.: Mitochondrial complementation and cytoplasmic male sterility in wheat. Genetics **63**, 611–618 (1969).

Taylor, D. L.: Chloroplasts as symbiotic organelles. Intern. Rev. Cytology **27**, 29–64 (1970).

Tipton, C. L., Mondal, M. H., Uhlig, J.: Inhibition of the K^+ stimulated ATPase of maize root microsomes by *Helminthosporium maydis* race T pathotoxin. Biochem. and Biophys. Res. Comm. **51**, 725–728 (1973).

Turbin, N. V., Atrashenok, N. V., Palilova, A. M., Lyulkina, E. I.: Mitochondrial structure in ovules of hybrid corn obtained by crossing sterile lines with fertility restoring lines. Doklady Biol. Sci. **182**, 536–538 (1968).

Turner, M. T., Martinson, C. A.: Susceptibility of corn lines to *Helminthosporium maydis* toxin. Plant Disease Reporter **56**, 29–32 (1972).

Ullstrup, A. J.: The impacts of the southern corn leaf blight epidemics of 1970–1971. Ann. Rev. Phytopathology **10**, 37–50 (1972).

Received August 8, 1974

Communicated by R. Riley

D. H. P. Barratt
R. B. Flavell
Cytogenetics Department
Plant Breeding Institute
Cambridge DB2 2LQ (England)